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Title: STUDIES OF THE HOST-PARASITE RELATIONSHIPS OF THE "SALMON POISONING" TREMATODE- NANOPHYETUS SALMINCOLA (CHAPIN)[1]

Abstract approved: Redacted for privacy
Dr. Raymond E. Millemann

The effect of experimental infection with the "salmon poisoning" fluke, Nanophyetus salmincola, on five species, including three subspecies, of salmonid fish was studied. The estimated numbers of cercariae that killed in 24 hours 50% of kokanee (Oncorhynchus nerka), Montana black-spotted cutthroat trout (Salmo clarki lewisi), Atlantic salmon (S. salar), coho salmon (O. kisutch), rainbow trout (S. gairdneri), and coastal cutthroat trout (S. clarki clarki), all between 30 and 37 millimeters in fork length, were 58, 74, 110, 200, 295, and 430, respectively. This range in sensitivity of the different species of fish to the parasite was correlated with the natural duration of the host-parasite relationships. The symptomatic, gross and histopathologic changes associated with penetration and migration of N. salmincola cercariae in the above trout and salmon species and in Lahontan cutthroat trout (S. clarki henshawi) are described. Penetration and migration of cercariae in fish were

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studied. Parasites were found inside fin rays and blood vessels, and were present in internal organs within 1 hour after epidermal penetration. Effects of different salinities on emergence, viability, and infectivity of N. salmincola cercariae were determined. Cercariae emerged from the snail, Oxytrema silicula, in waters having salinities that ranged from 2 to 20 ppt. Cercariae survived the longest (68 to 75 hours) in waters having salinities from 2 to 6 ppt. Cercariae penetrated fish at all test salinities (12 to 20 ppt) except at 4 and 8 ppt. Observations were made on emergence of cercariae from snails. Cercariae were present in the blood of the afferent ctenidial sinus. The parasites passed down the ctenidial leaflet through a central sinus to the leaflet apex and then into the mantle cavity.

Studies on the host-parasite relationships of the "salmon poisoning" trematode--*Nanophyetus salmincola* (Chapin)

by

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INTRODUCTION

Evidence for the pathogenicity of the "salmon poisoning" fluke, *Nanophyetus salmincola*, to fish is inconclusive. Ward and Mueller (1926) examined brook trout fry that had died in an Oregon hatchery during a severe epizootic. The fish were heavily infected with encysted metacercariae of a digenetic trematode later identified as *N. salmincola*. The above authors believed that the parasites were the cause of the losses. They found a direct relationship between the degree of popeye (exophthalmia) and the number of parasites in the optic nerves. Simms, Donham and Shaw (1931) and Simms *et al.* (1931) believed that fish could tolerate large numbers of parasites; e.g. they found 14,062 parasites in an apparently healthy naturally infected cutthroat trout 4.6 inches long. Simms (1933) reported heavy *N. salmincola* infections in young rainbow and brook trout that were dying in an Oregon hatchery. Exophthalmia was observed in many of the fish. Simms (1933) also found heavy infections in apparently healthy fish from streams and hatcheries. He reported that there were no deaths or signs of infection in young rainbow and brook trout that had heavy infections after laboratory exposure to infected snails. Shaw *et al.* (1934) exposed young rainbow and brook trout, approximately 2 inches long, to infected snails. The fish remained in good condition for periods up to 90 days even though some of the fish were exposed for 55 days to snails and were

heavily parasitized. Bennington (1951) and Bennington and Pratt (1960) concluded, on the basis of results from laboratory experiments in which fish and infected snails were placed together in aquaria, that the parasite is pathogenic. In these experiments, 12 chinook salmon, 2 dace, 1 sculpin, and 2 goldfish died 6 to 24 hours after exposure to infected snails. Bennington (1951) stated that the parasite "may be quite pathogenic to young fish" during the season of low water in streams having high populations of infected snails. Farrell and Lloyd (1962) reported that rainbow trout were killed after laboratory exposure to infected snails. They stated that the degree of pathogenicity was directly proportional to the rate of accumulation of cercariae by fish. All of the above studies were concerned with approximately 24 hour mortalities of fish. There is no information on long-term mortality of fish infected with N. salmincola.

Regarding the symptomatic, gross and histopathologic changes associated with the infection of fish, Ward and Mueller (1926), as noted above, believed that the parasites caused exophthalmia. Bennington and Pratt (1960) and Farrell and Lloyd (1962) were unable to produce this condition experimentally. Bennington and Pratt (1960) reported that a local dermatitis developed in salmon where cercariae had penetrated. They also observed extensive lesions of the fins, gills, tails, retinas, and corneas of wild infected fish. They concluded that these effects were produced by migrating cercariae because histological sections contained numerous metacercariae adjacent to the lesions. They stated that the metacercariae were

not harmful to fish. Wood and Yasutake (1956) studied the histopathology associated with the metacercariae in naturally infected hatchery-raised yearling coho salmon. They found little tissue reaction or inflammation around the parasites. Hyperplasia of epithelial cells or fibrous walls were observed around some parasites. There was evidence of "marked obstructive and mechanical injury" to the muscle fibers of the heart ventricle, the retina, the kidney tubules, the pancreatic tissue, and the gall bladder wall. Metacercariae observed in the aorta were considered large enough to obstruct this vessel. Wood and Yasutake (1956) concluded that an infected fish is weakened physiologically in practically every organ.

Simms, Donham and Shaw (1931) concluded on the basis of indirect evidence that cercariae entered the fish through the gills. Sinitsin (1930) stated that most of the cercariae attached to the fish's abdomen and moved along it to the urinary aperture and thus reached the kidney by this route; however, it is not clear whether he was observing the cercariae of N. salmincola or those of some other trematode. Bennington and Pratt (1960) found that few cercariae crawled on the skin or through the urinary aperture and that any part of the fish could be penetrated, the point of entry depending on the point of contact. Penetration took place within 30 seconds to 2 minutes; the cercariae then rested for a short time, and then migrated into deeper tissue. Bennington and Pratt (1960) also reported that cercariae that had penetrated into the tail of a fish entered blood vessels between the rays and crawled caterpillar-like to the caudal peduncle. They suggested that the parasites reached the kidney via the renal portal

system.

I have developed methods for maintaining infected snails during all months of the year, stimulating infective cercariae to emerge from snails, exposing fish to known numbers of cercariae, and determining the total number of encysted metacercariae in small fish. Thus, I have been able to study the effects of known numbers of parasites on the fish host under controlled conditions. Such studies have not been done previously.

Oxytrema silicula, the snail host for N. salmincola, occurs predominantly in the freshwater parts of streams. Gebhardt (1966) was the first to report the occurrence of the snail in brackish water. He collected snails from the Alsea, Siletz, and Yaquina Rivers in western Oregon during all seasons of the year. The maximum and minimum salinities recorded by him at the farthest points downstream in which he found snails were 4.2 and 0.8 ppt, 4.2 and 0.4 ppt, and 11.2 and 4.2 ppt in the Alsea, Siletz, and Yaquina Rivers, respectively.

Karpevich (in Zhadin, 1965) stated that molluscs can be adapted to tolerate salinities greater than those that occur in their normal habitat. He studied three species of the bivalve, Dreissena. One species was found exclusively in freshwater, whereas, the other two occurred in brackish water. D. polymorpha, which is found in freshwater of the Volga River died when the salinity was increased to 6 or 7 ppt. D. andrusovi and D. caspia, which are found usually in waters with salinities from 4 to 11 ppt (Caspian Sea), could not tolerate freshwater or waters with salinities above 17 ppt.

Published studies on emergence of cercariae from snails in waters

with different salinities, and on the salinity tolerance of cercariae have been done using only cercariae from marine snails. Rees (1948) studied the effect of different dilutions of seawater on emergence of Cercaria purpurae from the marine snail Nucella lapillus. Snails were held for 24 hours in water having a salinity of 35 ppt and were then placed in water having a salinity of 30 ppt for 24 hours. At the end of each succeeding 24-hour period snails were placed in waters of decreasing salinities at 26, 21, 17, and 13 ppt. Cercariae stopped emerging from snails when the salinity of the water was decreased to 17 ppt. The snails died after 12 hours in water at 13 ppt salinity.

Stunkard and Shaw (1931) studied the effect of different dilutions of seawater and tap water on the longevity of the following species of marine cercariae: Cryptocotyle lingua and Cercaria parvicaudata from the marine snail Littorina litorea; Cercariaeum lintoni, Cercaria quissetensis and Cercaria variglandis from Nassa obsoleta; and Cercaria sensifera from Urosalpinx cinereus. Most of the cercariae of all species died within 1 hour in tap water. In one-eighth seawater, cercariae were active for considerable periods of time and some of them were swimming after 4 hours. Cercariae were only slightly affected when held in one-fourth seawater.

Sindermann and Gibbs (1953) determined the salinity tolerance of Microbilharzia variglandis, a parasite of the marine snail Nassa obsoleta. Cercariae survived salinities to 3.2 ppt using different dilutions of seawater. Fifty per cent of the cercariae were dead in 12 hours in water at 12 ppt salinity. Some cercariae survived for more than 1 hour in distilled water. There have been no published

studies on cercariae emerging from freshwater snails in brackish water. Shaw (personal communication) in 1930 exposed N. salmincola cercariae, obtained from crushed snails, to seawater and to various concentrations of sodium chloride. All cercariae in undiluted seawater died immediately. At 20, 12.5, and 8.3 ppt, all cercariae were dead at 2, 7, and 45 hours, respectively. All control cercariae in tap water at room temperature were dead at 41 hours.

This study was undertaken to determine: (1) the effects of experimental infection with N. salmincola on five species, including three subspecies, of salmonid fish with respect to both short-term and long-term mortality, and symptomatic, gross and histopathologic changes associated with cercarial migration, and the routes of migration of cercariae in fish; (2) if cercariae will emerge from snails held in waters of different salinities; (3) the effect of different salinities on the viability and infectivity of cercariae; (4) the route of emergence of cercariae from the snail.

MATERIALS AND METHODS

I. Effects of N. salmincola cercariae on salmonid fishes under experimental conditions.

Snail culture

Two- to three thousand snails (O. silicula) were collected from the Big Elk River, 3.5 miles east of Elk City, Oregon, between July 7, 1965 and December 1, 1965. The mean maximum diameter of the shell aperture of 263 of these snails was 8.7 millimeters and the range was 6.6 to 10.8 millimeters. Snails were kept in plastic trays 4 feet long, 2 feet wide, 6 inches deep, containing chlorine-free tapwater at 17 to 18°C. The trays were subdivided into nine compartments each containing 70 snails and each provided with a continuous supply of air. Fresh water was added to the trays weekly to compensate for evaporation. The snails were fed dried alder leaves previously leached in hot tapwater for 8 hours, and chicken mash pellets consisting of ground mixed cereal grains. Leaves were added weekly and the pellets were sprinkled lightly into each compartment every 3 to 4 days. Broken clam shells and sand were added to provide a source of calcium and other minerals.

Under the above conditions, most of the snails infected with N. salmincola were maintained for 1 year from the date of collection. The degree of infection in the snails did not decline appreciably, and mature cercariae were obtained during all months of the year. Infected and noninfected snails laid egg masses from December 15, 1965 to February 1, 1966 and from March 1, 1966 to August 1966. Some of

these eggs were saved and kept in jars containing aerated tapwater at 17 to 18°C. The eggs hatched approximately 10 days later and these snails lived for 6 months.

Fish procedures

Fish representing five species of salmonids, 29 to 42 millimeters in fork length were obtained from hatcheries from September 23, 1965 to March 22, 1966. Atlantic salmon (Salmo salar), Lahontan cutthroat (S. clarki henshawi), and kokanee, which are landlocked sockeye salmon (Oncorhynchus nerka), were obtained from the Wizard Falls Hatchery in Oregon; rainbow trout (S. gairdneri) from the Klamath Hatchery in Oregon (these fish were hatched from eggs obtained from brood stock maintained at the Roaring River Hatchery in Oregon); Montana blackspotted cutthroat trout (S. clarki lewisi) from the U. S. National Fish Hatchery at Creston, Montana; coastal cutthroat trout (S. c. clarki) from the Cedar Creek Hatchery in Oregon; and coho salmon (O. kisutch) from the Alsea River Salmon Hatchery in Oregon. All hatcheries except the last two and the Roaring River Hatchery are located outside the enzootic area of the "salmon poisoning" fluke. Fish were obtained soon after hatching from the last two hatcheries during the time of year when cercariae of N. salmincola are not emerging from snails in streams. In all cases, 10 fish of each species were examined by the squash method described below for natural infection before others of the same species from the same lot were used in an experiment. Natural infections were not found in any fish, but an infection was found in a coastal cutthroat trout; it had two parasities. This fish, a part of the control group in

Experiment 3, was apparently accidentally infected in the laboratory. Stock fish were maintained in 620-gallon circular tanks of wood or in plastic trays. The tanks or trays were provided with flowing chlorine-free tapwater at temperatures from 6 to 10°C and at pH from 7.0 to 7.8. The pH was adjusted by bubbling carbon dioxide through the water before it entered the tanks. The pH of the incoming water was determined daily. Dissolved oxygen content, maximum and minimum temperatures, and pH of the tank water were recorded weekly. Oregon moist pellets (small size) manufactured by Bioproducts in Warrenton, Oregon, were fed to the fish once each day.

Collection of cercariae

In addition to N. salmincola, O. silicula can be parasitized by at least fiwe other species of digenetic trematodes (Burns, 1961), the cercariae of which, however, do not penetrate fish (Bennington and Pratt, 1960). Mixed infections involving N. salmincola are rare. Bennington (1951) examined 100 snails and found no mixed infections. I examined approximately 350 snails and found no mixed infections. Gebhardt (1966) reported only 15 of 1695 snails to have mixed infections. Only N. salmincola cercariae emerged from the snails in my experiment during the entire study period. The cercariae of N. salmincola are easily recognized. The identification, as N. salmincola, of the cercariae I used to infect fish was verified by microscopic examination during the counting procedure described below.

Twelve hours before cercariae were needed for an experiment, 200 to 300 infected snails were removed from the trays and washed in tapwater. Each snail was then placed in a 60 x 20 millimeter Petri

dish with 15 milliliters of tapwater at 20 to 22°C under ceiling light. The pH of the water had been adjusted previously from approximately 10.0 to 7.0 to 8.0 by continuous aeration for 12 hours. Light did not appear to stimulate emergence of cercariae, because in preliminary experiments cercariae emerged equally well when the snail is held either in darkness or approximately 12 inches from a fluorescent light. Most of the cercariae began emerging 3 to 4 hours after isolation, and 25% to 30% of the snails produced large numbers of cercariae during the 12-hour isolation period and only these cercariae were used in an experiment. Cercariae were pooled after 12 hours by pouring the water from the Petri dishes into a 1,000 milliliter beaker. Thirty minutes later the parasites were recovered from the bottom of the beaker.

Infection of fish---mortality studies

All of the fish species mentioned previously in the section Fish procedures, except Lahontan cutthroat, were used in this phase of the study. Sixty to 90 fish of one species were placed in a 5-gallon jar containing 5 gallons of tapwater at 10°C. The temperature of the water was raised to 20°C by the addition every 10 minutes of 100 milliliters of boiling water during a 1 hour period. Each fish was then placed into a separate 4 ounce paraffin-coated paper cup which contained 30 milliliters of pH-adjusted tapwater at 20°C and at pH of 7.3 to 7.8. The water was aerated continuously during the exposure period. Pooled cercariae were pipetted into Petri dishes so that separate small drops of water were formed. The cercariae in each drop were counted under 10X magnification. A known number of

cercariae, 50 to 1,000, were then washed into the cups containing the fish, and the fish exposed for 1 hour at 20°C which was a convenient laboratory room temperature. Ten fish were used for each exposure level and 50 to 70 fish in each experiment. Control fish were subjected to the same conditions except that cercariae were removed from the water to which the controls were exposed, by filtration through No. 41 Whatman filter paper. After exposure, the water from the cups used for the exposure at each level was pooled and examined for cercariae. Usually no more than 10 living and 20 to 30 dead cercariae (the number depending on the exposure level) were found. The fish in each experiment were of the same age and approximately the same fork length (Table I). The length was obtained from fish that died during the first 24 hours of the 30-day experiments.

Fish from each exposure level were pooled after exposure and placed in 1-gallon glass jars containing 1 gallon of continuously aerated tapwater in a 16°C constant temperature room. The dissolved oxygen, pH, and temperature of the water were determined when the fish were placed in the jars and whenever fish died. The pH values ranged from 7.3 to 7.9, and the dissolved oxygen values ranged from 8.1 to 10.1 milligrams/liter. The fish were fed Oregon moist pellets daily approximately 1 hour before the water in each jar was changed. Fish were observed for signs of infection every 6 to 8 hours during the first 24 hours after exposure and thereafter daily. Deaths of fish were recorded at these times, using cessation of respiratory movements as the criterion of death. The total number of encysted metacercariae in experimental fish that died between 24 hours and

30 days or were killed after 30 days was determined by squashing the fish between two 3 inch x 4 inch glass plates. The plates, held together by four small "C" clamps, were placed over a grid under 15X magnification and the parasites counted. Control fish were similarly examined.

The 24-hour mortality percentages were plotted against the exposure levels on semilogarithmic paper. The 24-hour lethal exposure level (24-hour LEL_{50}--the exposure level, in numbers of parasites, lethal to 50% of the fish in 24 hours) was determined for each species by interpolation after fitting a sigmoid curve by eye to all of the experimental data. The average number of parasites in fish that died between 24 hours and 30 days and in fish that were killed at the end of 30 days following exposure to each tested level was plotted against the 24-hour per cent mortality for the same exposure level. The 24-hour lethal dosage level (24-hour LD_{50}--the infection level, in numbers of parasites, that results in 50% mortality of the fish in 24 hours) was estimated from these curves for each species.

Parasites were not counted in fish that died within the first 24 hours. During this time cercariae were not encysted and their excretory bladders were not filled. Thus, they were not clearly visible for counting by the squash technique. They were also too fragile to be counted by the homegenization-sedimentation technique outlined in Gebhardt et al. (1966). Therefore, I have assumed that the number of parasites in fish that survived for the first 24 hours approximates the number present in fish that died during this time.

Penetration and migration of cercariae in fish; histopathology

1. Percutaneous exposure

Initial penetration of cercariae into fish was studied by placing cercariae on the caudal fin of intact pithed fish (Lahontan cutthroat trout) and on 1 centimeter sections of caudal fin clipped from pithed Lahontan cutthroat trout, and then observing penetration under 15X magnification. Subsequent migration of the parasites in fish and the histopathologic changes associated with this migration were studied by examining histological sections. Each of six Lahontan cutthroat trout, 42 to 60 millimeters in fork length, was exposed to 500 cercariae in the manner previously described. One fish was killed at each of the following times after exposure: 15, 30, 60, 120, 720, and 1440 minutes. The fish were fixed for 24 hours in Mossman's AFA fixative. A cross-sectional cut was made through the body of each fish at the anterior edge of the dorsal fin and a second cut was made 1 centimeter posterior to the first. This block of tissue was mounted in paraffin and sections were cut at 15 microns. Every tenth section of the ribbon was stained with Harris' alum hematoxylin and eosin. Twenty-seven sections were examined from each exposed fish and 3 to 4 sections from a nonexposed control fish.

2. Alimentary canal exposure

Each of ten Lahontan cutthroat trout 60 millimeters long and each of eight rainbow trout 110 to 150 millimeters long was exposed to 100 and 500 cercariae, respectively, by stomach tube, using a 2 millimeter inside diameter nylon tubing attached to a 2 milliliter hypodermic syringe. Two control cutthroat trout were used to test

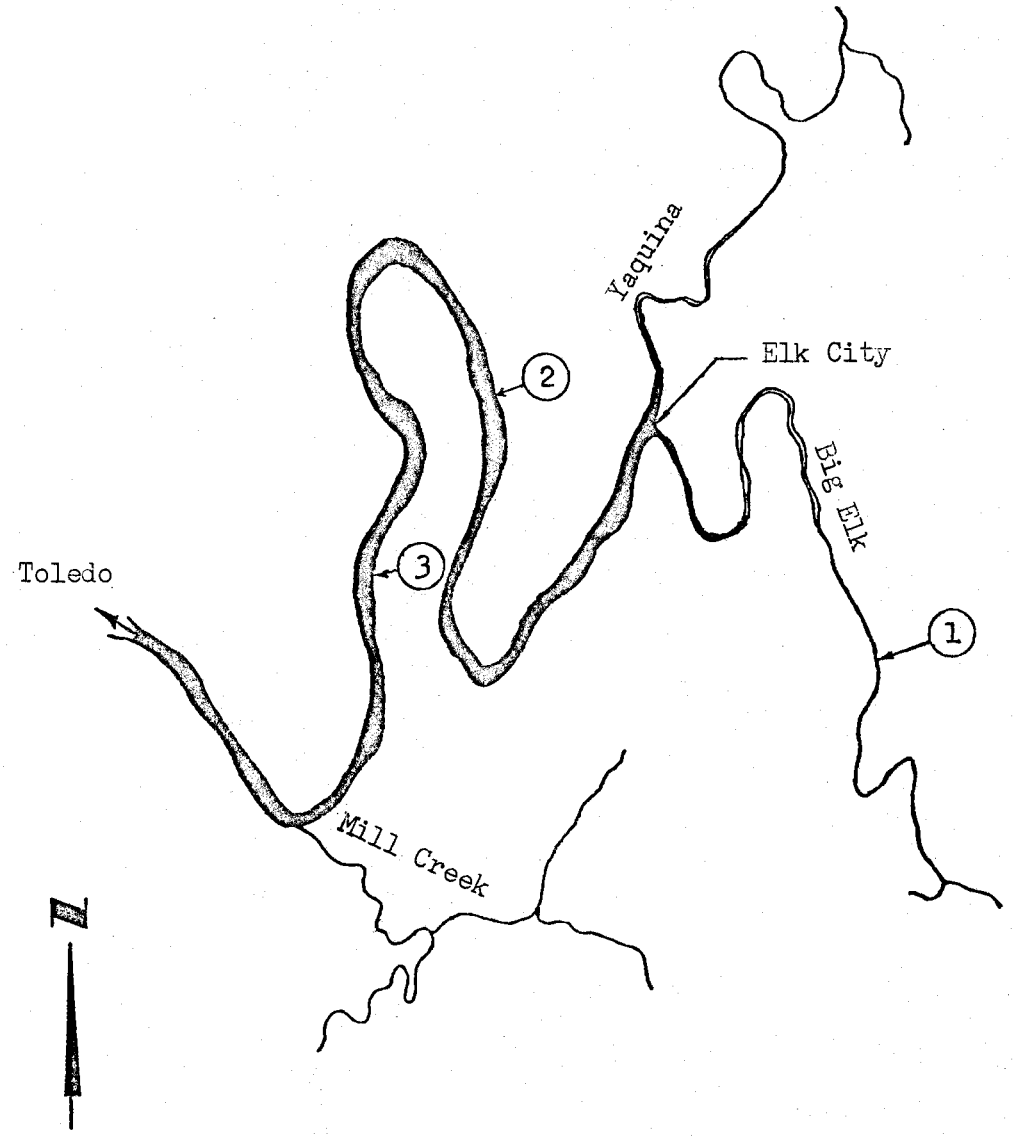
for infectivity of the cercariae and were exposed percutaneously. Another group of five rainbow trout 150 millimeters long was exposed to unknown numbers of cercariae by stomach tube on three different days during a period of 6 days. Also, each of six Lahontan cutthroat trout 60 to 70 millimeters long received by stomach tube two cubic sections 2 to 3 millimeters on a side of infected snail ovarian tissue. Ten control fish received non-infected ovarian tissue.

To test for infectivity of the parasites, 700 48-day-old metacercariae were recovered from fish that had received free cercariae by stomach tube. These were given to each of two hamsters by stomach tube. The animals were killed 14 days later and the small intestine examined for adult flukes.

II. Emergence of N. salmincola cercariae from snails held in waters of different salinities.

Snails were collected from brackish water at two locations on the Yaquina River in western Oregon. These sites, areas 2 and 3, were 2.5 and 5 miles, respectively, downstream from Elk City, Oregon (Figure 1). High and low tide salinities at area 2 was 9.4 and 2.2 ppt on September 8, 1965, and at area 3 was 20.5 and 4.1 ppt on August 25, 1965. Between 150 and 200 snails were collected at area 2 and 300 snails were collected at area 3. Snails infected with N. salmincola were separated from those infected with other species of trematodes and were maintained in a plastic holding tray containing diluted seawater at 4 ppt salinity.

Infected snails were isolated individually in 60 x 20 millimeter Petri dishes containing tap water that had the pH adjusted previously



Scale: 1 inch = 1 mile

Figure 1. Map of part of the Yaquina River system drawn from the United States Geodetic Survey map of the Toledo quadrangle. The circled numbers designate snail collection sites. The head of tidewater is located at the junction of shaded and unshaded parts of the river.

from approximately 10.0 to 7.0 to 8.0 by continuous aeration for 12 hours. The freshwater was replaced with seawater diluted with pH-adjusted tapwater to give a salinity of 4 ppt at the end of 24 hours. At the end of each succeeding 24-hour period the seawater was replaced with seawater of the next highest test salinity. The tested salinities were: 4, 8, 12, 14, 16, 18, and 20 ppt prepared from seawater having a known salinity using pH-adjusted tapwater. A 10 millimeter disc of lettuce was placed in each dish for snail food. The experiments were done in a 20°C room. Cercariae were counted either directly or by computing an average based on five 1.0 milliliter aliquots taken from a homogenous suspension of cercariae.

III. Effect of different salinities on viability and infectivity of N. salmincola cercariae.

Viability of cercariae

Infected snails obtained from freshwater (Figure 1, area 1) and 10 snails from each of the brackish water areas (Figure 1) were isolated in individual 60 x 20 millimeter Petri dishes. The former were held in 15 milliliters of freshwater, and the latter in 15 milliliters of seawater at 10 ppt salinity. The dishes were examined every 4 hours for cercariae. Cercariae, 4 hours old or less, from snails from each area were pooled and divided into equal groups of 15, 20, or 25 in a drop of water in 60 x 20 millimeter Petri dishes. Ten milliliters of freshwater or seawater of a given salinity, from 1 to 20 ppt, were added to each dish. Salinities higher than 20 ppt were not tested because this was the highest salinity recorded at collection site 3. The dishes were examined immediately after the water

was added and thereafter every 4 to 7 hours for dead cercariae. Cessation or movement and lack of response to vibration, tapping of the dish, were used as criteria of death. The experiments were terminated when more than 50% of the cercariae were dead in each dish.

Infectivity of Cercariae

The cercariae used in these tests were those that emerged from snails collected from the sites in brackish water. The snails were held in freshwater and in seawater at the different test salinities described above. Cercariae that emerged during each 24-hour period from snails of one area were pooled and tested for infectivity by one of three methods. Cercariae that emerged in freshwater were exposed to living Lahontan cutthroat trout (45 to 50 millimeters in fork length) in freshwater. Approximately 400 cercariae from area 2 were placed into each of three cups, each cup containing one fish. These fish were exposed percutaneously in the manner previously mentioned. The second method involved those cercariae that emerged at 4 and 8 ppt salinity. These cercariae were exposed to a fish from brackish water, the three-spine stickleback (Gasterosteus aculeatus microcephalus) obtained from Eckmann Lake, Oregon. It was necessary to use this salinity-tolerant fish because salmonids adapted to saltwater were not available. Gebhardt et al. (1966) have shown that the stickleback can be experimentally infected with N. salmincola. The test sticklebacks were assumed not to be naturally infected because examination of some from the same lot used in the experiment were negative for N. salmincola metacercariae. The third method involved those cercariae emerging at 12, 14, 16, 18, and 20 ppt salinity. These

parasites were exposed to pithed, intact Lahontan cutthroat trout in a 90 x 15 millimeter Petri dish. One fish was used for each test salinity and for each snail collection area. Penetration of the cercariae into the caudal fin of the fish was observed microscopically. I found that the stickleback was not as susceptible as salmonids to the parasite. Thus, it was necessary to use a pithed salmonid to test for the infectivity of the small numbers of cercariae emerging at the higher salinities.

IV. Emergence of N. salmincola cercariae from the snail, O. silicula.

A group of snails were placed in individual Petri dishes to allow cercariae to emerge. One dish in which large numbers of cercariae were being produced was selected for examination and removed to a new dish of fresh tapwater for observation. The snail was held with fingers by the apical end in the dish of water with the aperture upward and the apex to the left. The snail was observed at 7x magnification under a dissection microscope. After the snail had extended its head and foot to the bottom of the dish, a strong light was directed into the mantle cavity for observation.

RESULTS

I. Effects of N. salmincola cercariae on salmonid fishes under experimental conditions.

Infection of fish--mortality studies

Most of the experimental fish exposed to large numbers of cercariae died within the first 24 hours (Figure 2). No control fish died during this time. Some experimental fish, but no control fish, died 1 to 2 days after exposure. Some experimental and control fish of all species, except kokanee, died within 3 and 30 days after the start of the experiments. There was no apparent difference between the numbers of experimental and control fish that died during this time (Table I).

I feel that the results of coastal cutthroat trout Experiment 3 are more reliable than results from Experiments 1 and 2, because 64% of the cercariae penetrated and encysted in Experiment 3, and only 43% in the other two experiments. The former figure is within the range (60% to 76%) of percentages recorded for the other species of fish. The use of defective cups in the first two experiments, in which cercariae may have been trapped in the paper fibers, could have been responsible for this difference. The data, therefore, from Experiments 1 and 2 are not shown in Figures 2 and 3, but are presented in Table I.

After exposure to 200 parasites, 100%, 90%, and 70% of the kokanee, Montana black-spotted cutthroat trout, and Atlantic salmon, respectively, died during the first 24 hours (Table I). Only one coho salmon and no rainbow trout or coastal cutthroat trout died

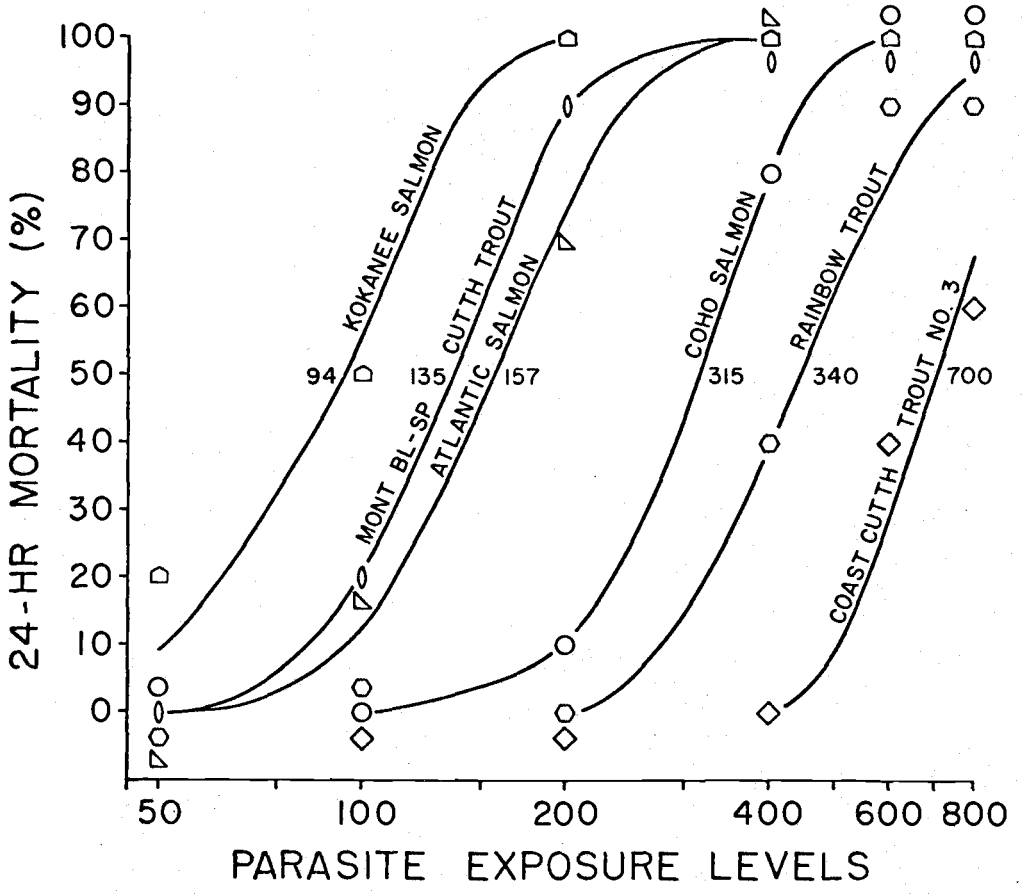


Figure 2. The 24-hour mortalities of salmonid fish exposed to known numbers of Nanophyetus salmincola cercariae, in relation to the exposure levels. The number beside each curve is the LEL_{50} value for that species of fish. Mont Bl-Sp Cutth Trout = Montana Black-Spotted Cutthroat Trout. Coast Cutth Trout = Coastal Cutthroat Trout.

Table I. Results of experimental exposure of six species of salmonid fish to Nanophyetus salmincola.

Fish			No. parasites			No. fish dead						
						Days after exposure						
Species	Age (weeks)	Length (mm)	Used for exposure	Recovered Range	Mean	0-1	2	3	4-10	11-20	21-30	Totals
Kokanee	14	30	600	-	-	10	0	0	0	0	0	10/10
			400	-	-	10	0	0	0	0	0	10/10
			200	-	-	10	0	0	0	0	0	10/10
			100	50-68	58	5	1	0	0	0	0	6/10
			50	27-52	39	2	0	0	0	0	0	2/10
			0	0		0	0	0	0	0	0	0/10
Montana black-spotted cutthroat trout	17	31	800	-	-	10	0	0	0	0	0	10/10
			600	-	-	10	0	0	0	0	0	10/10
			400	-	-	10	0	0	0	0	0	10/10
			200	-	77	9	1	0	0	0	0	10/10
			100	56-79	67	2	0	0	0	0	4	6/9
			50	35-44	38	1	0	0	0	0	0	1/8
			0	0		0	0	0	0	4	1	5/9
Atlantic salmon	12	31	400	-	-	10	0	0	0	0	0	10/10
			200	145-162	154	7	0	0	0	0	0	7/10
			100	66-84	77	2	0	0	1	1	1	5/10
			50	32-43	37	0	0	0	1	1	0	2/9
			0	0		0	0	0	0	1	2	3/10
Coho salmon	2	37	800	-	-	10	0	0	0	0	0	10/10
			600	-	-	10	0	0	0	0	0	10/10
			400	275-335	305	8	0	0	0	0	1	9/10
			200	101-154	122	1	0	0	1	1	1	4/10
			100	50-98	72	0	0	1	1	1	0	3/10
			50	30-55	36	0	0	0	1	0	1	2/10
			0	0		0	0	0	2	3	4	9/10

Table I. (Continued)

Fish			No. parasites			No. fish dead						
						Days after exposure						
Species	Age (weeks)	Length (mm)	Used for exposure	Recovered Range	Mean	0-1	2	3	4-10	11-20	21-30	Totals
Rainbow trout	16	33	800	-	382	9	0	0	0	0	0	9/10
			600	-	444	9	0	0	0	0	0	9/10
			400	227-327	269	4	0	0	0	1	0	5/10
			200	67-185	115	0	0	0	2	0	2	4/10
			100	44-72	58	0	0	0	0	3	1	4/10
			50	15-49	31	0	1	0	2	0	1	4/10
			0	0		0	0	0	0	1	2	3/9
Coastal cut-throat trout Exp. #1	8	29	800	301-450	356	5	1	0	1	1	0	8/10
			600	136-256	186	2	0	0	2	1	1	6/10
			400	102-256	155	0	0	0	3	3	1	7/10
			200	61-156	88	0	0	0	2	3	0	5/9
			100	28-71	45	0	0	0	2	4	1	7/10
			50	15-44	27	0	0	0	2	4	1	7/10
			0	0		0	0	1	5	0	0	6/10
Coastal cut-throat trout Exp. #2	9	31	1,000	387-559	495	7	0	0	0	1	0	8/10
			800	196-384	298	7	0	0	0	0	3	10/10
			600	109-344	219	2	0	0	3	1	0	6/10
			400	107-291	180	1	0	0	4	1	1	7/10
			200	42-142	95	0	0	0	0	2	0	2/9
			0	0		0	0	0	1	1	0	2/10
Coastal cut-throat trout Exp. #3	12	31	800	422-531	467	6	0	0	1	2	0	9/10
			600	323-373	348	4	1	0	1	4	0	10/10
			400	203-378	276	0	0	0	4	5	0	9/10
			200	108-177	138	0	0	0	5	3	0	8/9
			100	48-88	68	0	0	0	5	2	1	8/10
			0	2		0	1	0	4	5	0	10/10

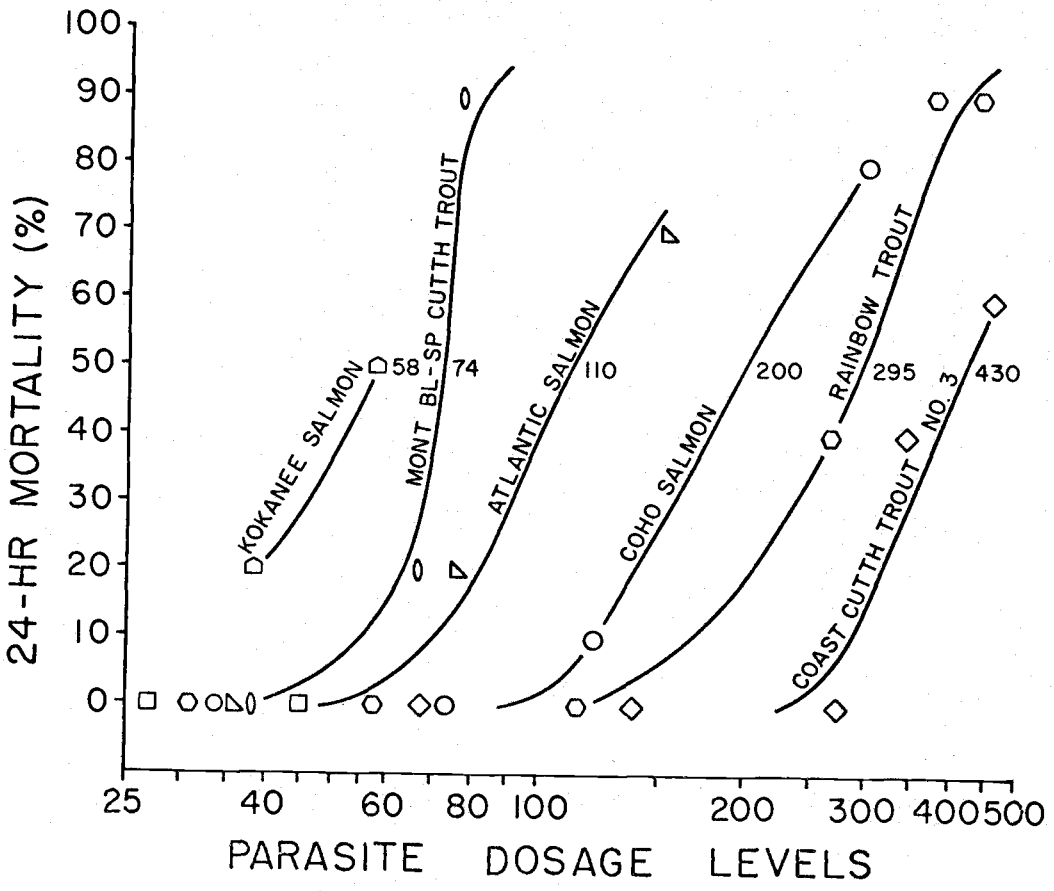


Figure 3. The 24-hour mortalities of salmonid fish infected with Nanophyetus salmincola metacercariae, in relation to estimated numbers of parasites. The numbers (average) of parasites in these fish were assumed to be the average numbers found in fish that were exposed to the same numbers of cercariae and that survived for the first 24 hours of the experiments. The number beside each curve is the LD_{50} value for that species of fish. Abbreviations same as for Figure 2.

during this time at this exposure level (Figure 2). All of the coho salmon and 90% of the rainbow trout died within 24 hours after exposure to 600 parasites, whereas a 40% mortality (Experiment 3) of the coastal cutthroat trout was observed (Figure 2). The 24-hour $LEL_{50's}$ for kokanee, Montana black-spotted cutthroat trout, Atlantic salmon, coho salmon, rainbow trout, and for coastal cutthroat trout (Experiment 3) are 94, 135, 157, 315, 340, and 700, respectively (Figure 2).

The highest parasite numbers in kokanee, Montana black-spotted cutthroat trout, and Atlantic salmon that survived for the first 24 hours were 68, 79, and 162, respectively (Table 1). One coastal cutthroat trout that survived had 559 metacercariae, and seven had between 400 and 550 parasites. Coho salmon and rainbow trout that survived had numbers of parasites less than those found in coastal cutthroat trout but much greater than those found in the other named species (Table 1). The highest number of parasites in coho salmon was 335, and one rainbow trout had 444 parasites. The 24-hour $LD_{50's}$ for kokanee, Montana black-spotted cutthroat trout, Atlantic salmon, coho salmon, rainbow trout, and for coastal cutthroat trout (Experiment 3) are 58, 74, 110, 200, 295, and 430, respectively (Figure 3).

The average percentages and ranges of percentages (in parentheses) of cercariae that penetrated and encysted in each fish species are as follows: kokanee 68% (58 to 78%); Montana black-spotted cutthroat trout 60% (38 to 76%); Atlantic salmon 76% (74 to 77%); coho salmon 70% (61 to 76%); rainbow trout 61% (48 to 74%); coastal cutthroat trout 64% (58 to 69%). These figures were calculated using

the numbers of parasites recovered from fish that survived for the first 24 hours of the experiment.

Penetration and migration of cercariae in fish.

1. Percutaneous exposure.

Cercariae penetrated the skin of the fish at any point and penetration was completed within 3 to 5 minutes. These observations are in agreement with those of Bennington and Pratt (1960), and contrary to statements by Sinitsin (1930) and Simms, Donham and Shaw (1931), who believed that the urinary aperture and gills were the portals of entry. I observed that cercariae became active when they were within 1 millimeter of the fish's skin. Skin penetration by the parasite involved attachment of the ventral sucker and contraction of the body into an egg-shaped mass. The anterior end was then forced into the skin. Cercariae migrated through the skin along a path parallel to the surface without the resting period mentioned by Bennington (1951). Cercariae that penetrated the caudal fin entered either blood vessels between rays, as first observed by Bennington and Pratt (1960), or blood vessels inside rays (Figure 4). They then migrated to the base of the fin. The circulatory system apparently is one of the principal routes used by cercariae to reach internal organs, such as the liver and heart, since parasites were seen adjacent to and inside blood vessels (Figures 5, 6, 7) in the hypodermis. Cercariae were seen in renal blood vessels; thus, they could reach the kidney, where they are found in large numbers, via the renal portal system, as suggested by Bennington and Pratt (1960). Cercariae were also observed in the peritoneum and body cavity and may enter internal organs from these

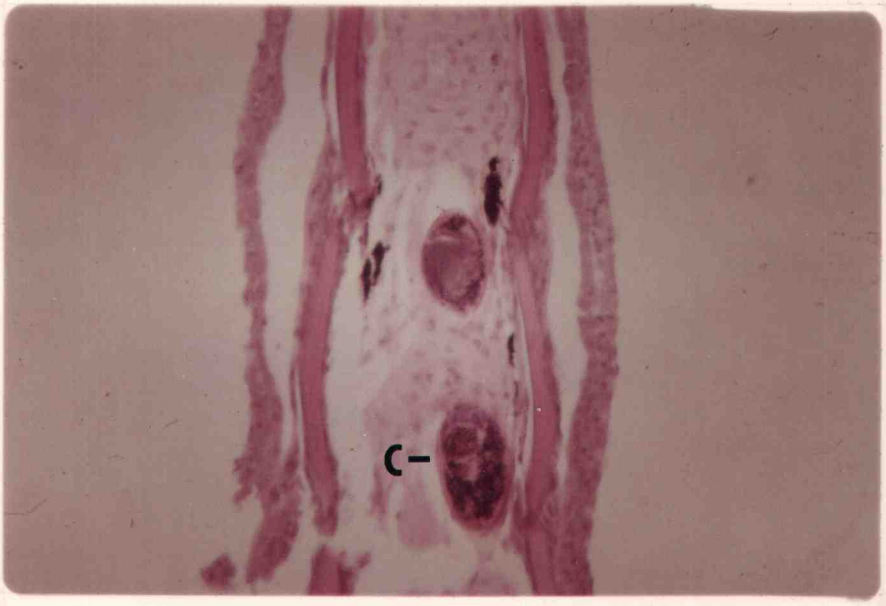


Figure 4. Cross section through the caudal fin of a Lahontan cutthroat trout, showing two cercariae (C) inside a fin ray. X 377.

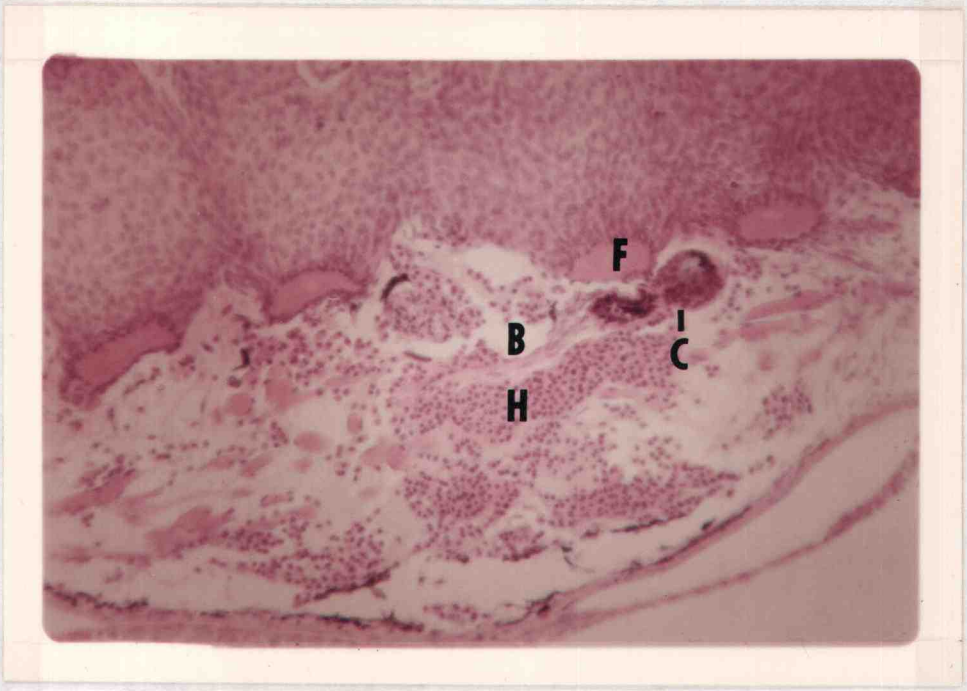


Figure 5. Cross section through the base of the caudal fin of a Lahontan cutthroat trout 52 millimeters long exposed to 500 cercariae of N. salmincola, showing hemorrhagic area (H) in loose connective tissue and a cercaria (C) near a fin ray (F) and a blood vessel (B). X 377.

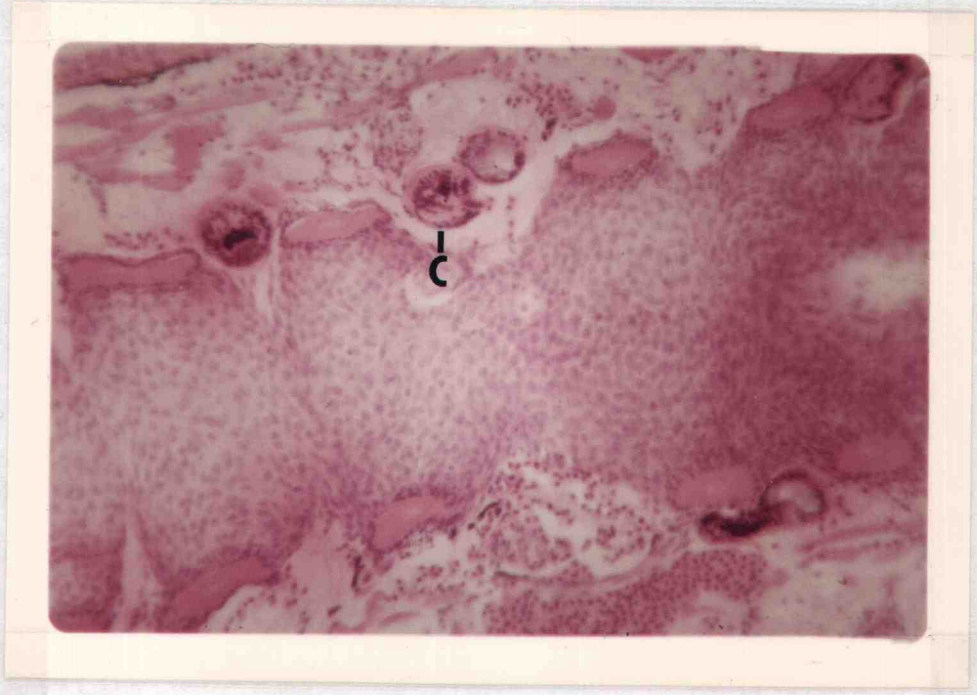


Figure 6. Cross section through the base of the caudal fin of a Lahontan cutthroat trout, showing one or two cercariae (C) of N. salmincola inside a blood vessel and two other cercariae in the subcutaneous tissue. X 377.

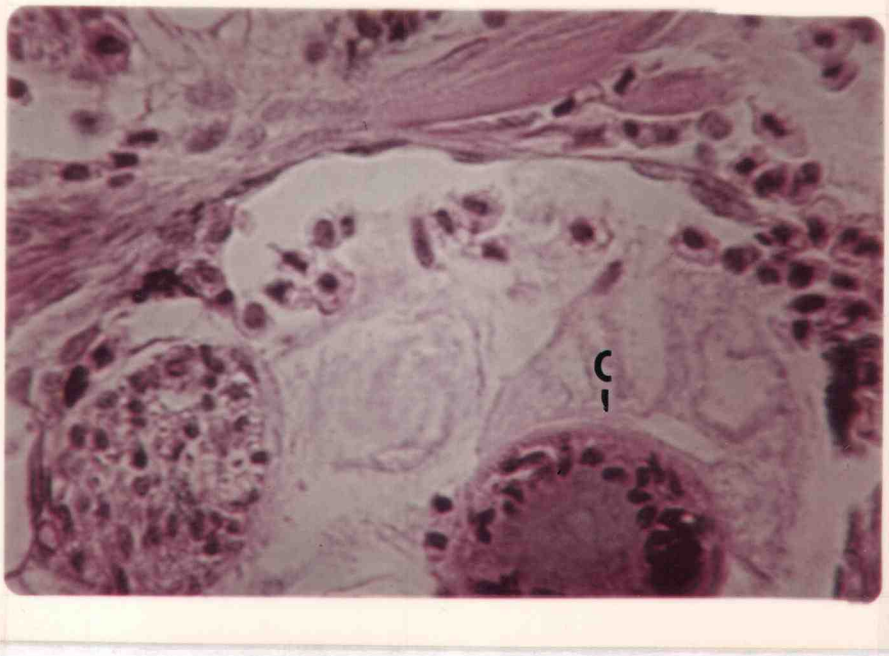


Figure 7. Higher magnification of Figure 6, showing cercariae (C) in a blood vessel. X 1525.

sites. Many cercariae were seen in the body musculature. These parasites probably do not migrate to internal organs, but rather, remain in the muscles, where they are found in large numbers. Many cercariae were found in the epidermis and hypodermis 15 and 30 minutes after exposure of fish, and by 60 minutes most of them were deep in the body musculature and some were seen in the kidney for the first time.

a. Symptomatic and pathologic changes

Gradations of the following signs of infection were observed in all infected fish but not in the controls. A decrease in swimming activity and an absence of the characteristic darting movement (except for Atlantic salmon, which characteristically remain at the bottom of an aquarium) appeared 1 to 2 hours after exposure. Biting was not common during the first 24 hours among infected fish. Diffuse petechiae on the body surface and larger hemorrhagic areas at the bases of the pectoral, pelvic, and caudal fins were observed within 2 hours (cf. Figure 8 and 9). Hemorrhagic areas reached a maximum size 12 hours after exposure of fish and many were visible for 48 hours. Equilibrium loss, drifting, erratic swimming, increased rate of respiratory movements, and in some fish vertical or horizontal tail curvature, were observed. These changes were most pronounced in fish that died within the first 24 hours, and were not always present in surviving fish. Papules were observed, on the skin of fish, presumably where cercariae penetrated. These were first reported by Bennington and Pratt (1960). Orbital hemorrhage was seen in many dead fish. There were large numbers of extravasated red blood cells

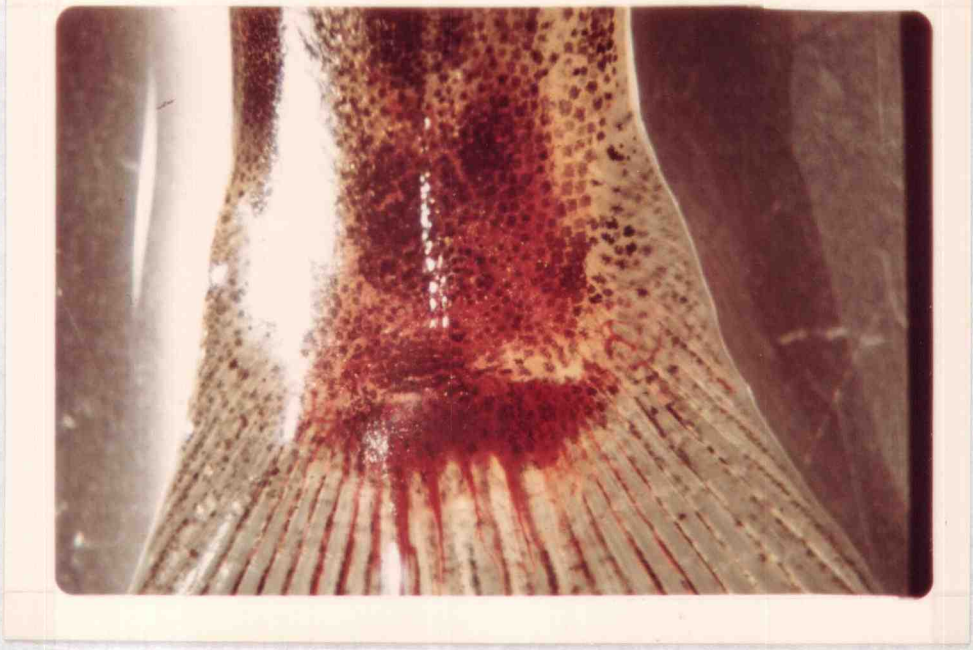


Figure 8. Photograph of caudal region of a Lahontan cutthroat trout exposed to 700 cercariae of Nanophyetus salmincola 24-hours before the picture was taken, showing large hemorrhagic area. X 22.

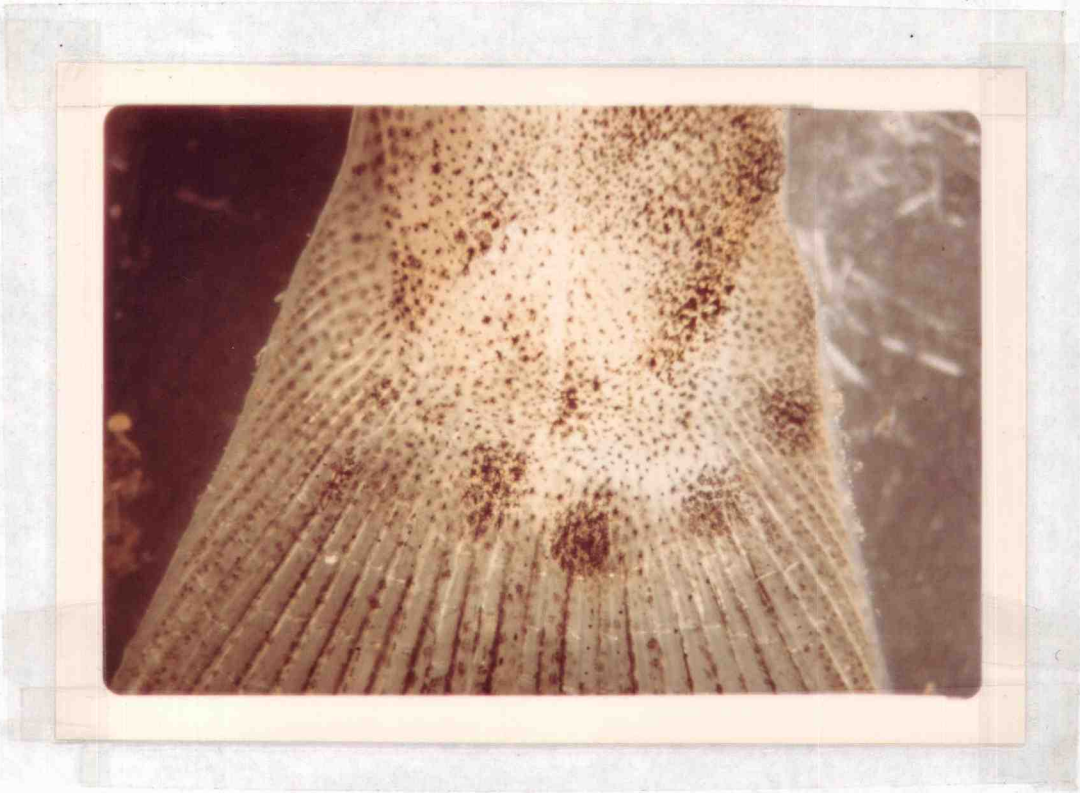


Figure 9. Caudal region of an uninfected Lahontan cutthroat trout 45 millimeters long. X 22.

in the loose connective tissue between the dermis and muscle bundles in the hemorrhagic area at the caudal fin base (Figure 5). The hemorrhage probably was due to destruction of the blood vessels by migrating cercariae. Destruction of muscle tissue posterior to a cercaria was observed and may have resulted from migration of the parasite. Partial renal tubule and blood vessel occlusion were observed adjacent to a parasite. This is the first detailed report on pathologic changes associated with penetration and migration of N. salmincola cercariae. Wood and Yasutake (1956) studied the histopathology associated with encysted metacercariae in naturally infected fish.

2. Alimentary canal exposure

The majority of fish that received either infected snail tissue or cercariae by stomach tube were infected. However, the number of metacercariae recovered from these fish was less than the number recovered from the percutaneously-exposed control fish. Parasites from the experimental fish were found in the heart, liver, kidney, intestinal wall, roof of the mouth, and gills. These parasites were infectious, as shown by recovery of adult flukes from hamsters that received metacercariae 14 days previously. The presence of metacercariae in internal organs of the experimental fish suggests that they may have developed from cercariae that reached these sites via the circulatory system or by penetration of the alimentary tract wall. These suppositions are in agreement with observations on histological sections of infected fish. I believe on the basis of the above findings that fish in nature may become infected

percutaneously and orally by ingestion of infected snails or free cercariae. Ching (1957) and Gebhardt (unpublished observation) reported the occurrence of snails in fish stomachs. Gebhardt (unpublished) has observed that coho salmon 46 millimeters long will become infected in the laboratory after avidly eating freely falling cercariae.

II. Emergence of N. salmincola cercariae from snails held in waters of different salinities.

There were no deaths of snails held at the various test salinities during the experimental period of 8 days. Cercariae emerged from snails from both brackish water areas at all of the test salinities except those snails from area 3 held at 8 ppt salinity (Table II).

There is an inverse relationship between increase in salinity and the number of snails from which cercariae emerged. Cercariae emerged from only one snail of ten from each area at 20 ppt salinity, and only a few cercariae emerged from these snails. However, even at relatively high salinities an appreciable number of cercariae emerged, e. g. a total of 1,500 cercariae emerged from two snails from area 2 at 16 ppt salinity. The results of this study show that cercariae can emerge from snails, and also that snails can live for several days, at salinities higher than those that the snails encounter in nature.

III. Effect of different salinities on viability and infectivity of N. salmincola cercariae.

Viability of cercariae

Cercariae from snails collected from both freshwater and

Table II. Effect of different salinities on emergence and infectivity of N. salmincola cercariae.

Snail source	Days	Salinity (ppt)	Total no. snails from which cercariae emerged of 10 initial	Total cercariae emerging (aliquot or actual count	Method of testing infectivity	Results of test[1]
Area 2	1	0	7	2,240	Exposed 400 cerc. to each of 3 fish (cutthroat).	187 cysts recovered
	2	4	7	30,000	Exposed all cerc. to 1 fish (stickleback).	No cysts recovered
	3	8	7	500	"	"
	4	12	7	1,500	Exposed all cerc. to pithed fish (cutthroat).	Observed penetration.
	5	14	4	250	"	"
	6	16	2	1,500	"	"
	7	18	2	165	"	"
	8	20	1	10	"	"
Area 3	1	0	2	100	Exposed all cerc. to 1 fish (cutthroat).	2 cysts recovered
	2	4	4	2,000	Exposed all cerc. to 1 fish (stickleback).	No cysts recovered
	3	8	0	0	----	----

1. Fish were examined for metacercariae by the homogenization-sedimentation technique outlined in Gebhardt, et al. (1966)

Table II. (Continued)

Snail source	Days	Salinity (ppt)	Total no. snails from which cercariae emerged of 10 initial	Total cercariae emerging (aliquot or actual count	Method of testing infectivity	Results of test[1]
	4	12	4	25	Exposed all cerc. to pithed fish (cutthroat).	Observed penetration
	5	14	1	10	"	"
	6	16	2	20	"	"
	7	18	5	20	"	"
	8	20	1	3	"	"

brackish water survived the longest (75 hours) at salinities between 2 and 6 ppt (Figure 10). Cercariae in freshwater had a shorter survival time (38 hours). At 12 ppt, one-half of the cercariae from snails collected from freshwater were alive after 4 hours, whereas, one-half of the cercariae from snails collected from brackish water were alive after 20.5, 26, and 38 hours. Cercariae from area 3 snails survived the longest (38 hours) at 12 ppt. At 16 ppt, one-half of the cercariae from snails from areas 2 and 3 were alive at 5, 6, and 9 hours. Cercariae from snails from freshwater survived less than 5 hours at 13 ppt salinity.

It appears that at lower salinities (2 to 6 ppt), cercariae from snails from the highest salinity collecting area did not do as well as cercariae from snails collected from freshwater. However, further experiments must be done to prove this conclusively.

Infectivity of Cercariae

Cercariae penetrated fish at all test salinities except at 4 and 8 ppt.

I have no explanation why cercariae did not penetrate fish at these salinities, but did so at higher salinities. A partial reason may be that a more susceptible species of fish (cutthroat trout) was used in the tests involving salinities greater than 4 ppt; whereas, the stickleback was used to test for infectivity of cercariae shed at 4 and 8 ppt salinity.

I have assumed that cercariae that penetrated fish in these experiments would have encysted; however, this was not determined in these studies.

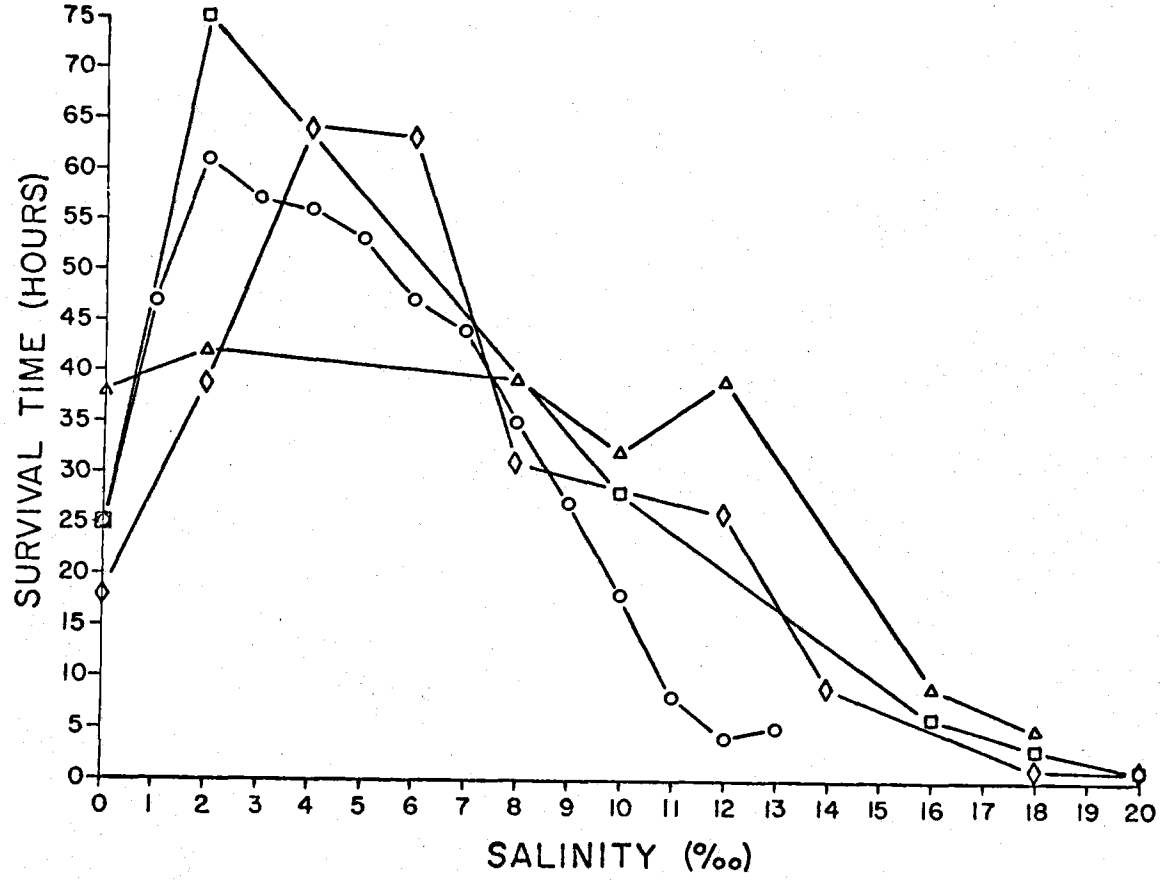


Figure 10. Survival times in hours of 50% of different groups of cercariae placed in fresh water and different concentrations of sea water (ppt). Cercariae came from snails collected from area 1 (O), area 2. (◊ and □), and area 3. (Δ).

IV. Emergence of N. salmincola from the snail, O. silicula.

Cercariae were carried rapidly in the blood inside the perivisceral, rectal, and mantle sinuses. These sinuses are connected to the afferent ctenidial sinus at the right margin of the ctenidium according to Ching (1957). Cercariae passed into the afferent ctenidial sinus and then to the central sinus of each ctenidial leaflet. At this time the cercariae suddenly stopped drifting. They orientated lengthwise in the central sinus and moved by alternate contraction and extension of the body to the ctenidial tip where they emerged, anterior end first, into the mantle cavity. The cercariae began to contract and extend rapidly, as soon as they were completely free of the ctenidial tip. They drifted out of the mantle cavity in the exhalent current of water on the right side of head (Figure 11). Bennington and Pratt (1960) stated that cercariae were released from snails in ropy strands of mucus. I never observed this, but I did find that cercariae after several hours became entangled in mucus secreted by the snails if the latter were confined in small vessels. No cercariae were ever seen emerging from the surface of the foot or head. However, histological sections revealed cercariae within the foot muscle.

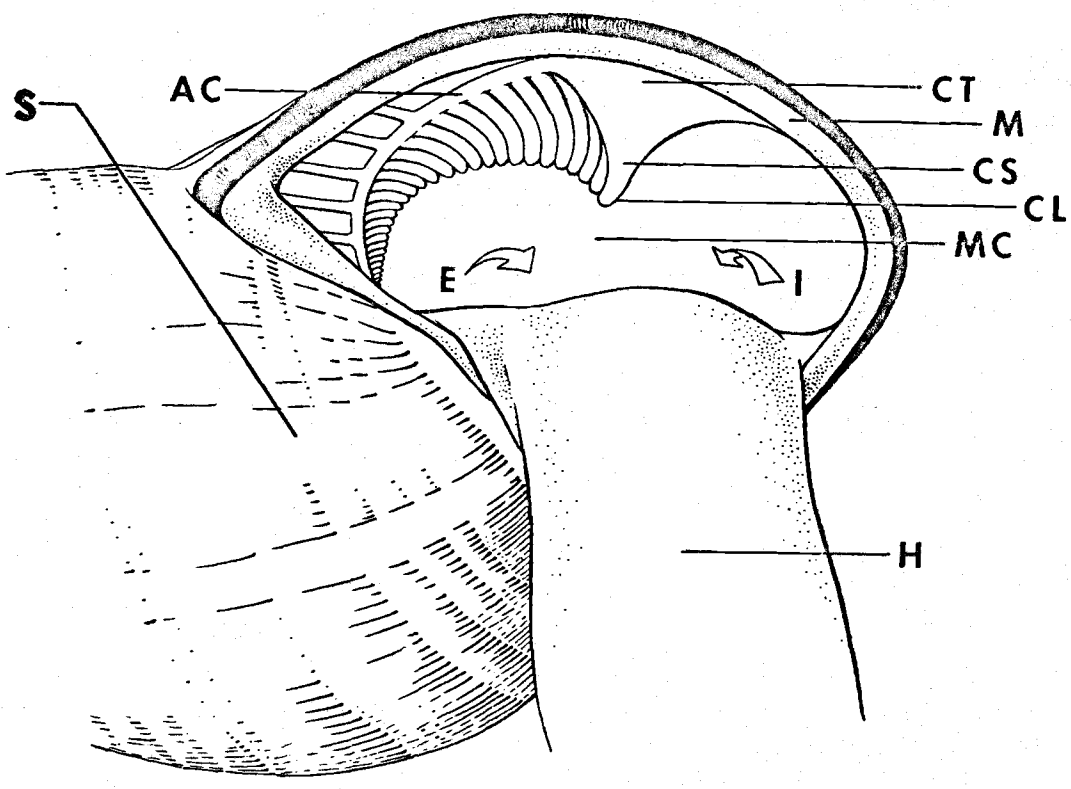


Figure 11. Mantle cavity of the snail, Oxytrema silicula. AC = Afferent ctenidial sinus, CL = Ctenidial leaflet, CS = Central sinus, CT = Ctenidium, E = Exhalent water current, H = Head, I = Inhalent, M = Mantle, MC = Mantle cavity, S = Shell.

DISCUSSION

This is the first report on the pathogenicity of N. salmincola for fish under controlled conditions involving known numbers of parasites. Deaths of experimental fish within the first 24 hours after exposure to cercariae are attributed to the effects of the parasites because no controls died during this time. Philip (1955) reported that the etiologic agent of "salmon poisoning" disease, Neorickettsia helminthoeca did not affect trout fingerlings. Thus, it is unlikely that the rickettsiae could have caused the death of the fish. Deaths of fish after the first 2 days of the experiments could not be definitely attributed to the parasites because controls also died. These fish may have died because conditions were not satisfactory for their maintenance in the laboratory. For example, aggression (fighting and biting), presumably a result of crowding, was common among the coho salmon, rainbow trout, and coastal cutthroat trout, and many had body and fin damage.

It is clear from my studies that there are large differences in sensitivity to the early stage of infection among the different fish species tested. The five species of fish can be divided into three groups having different relative sensitivities, as shown by the LEL and LD curves (Figures 2 and 3). The coastal cutthroat trout is the most resistant species. The rainbow trout and coho salmon are intermediate in resistance, and kokanee, Atlantic salmon, and Montana black-spotted cutthroat trout are the most sensitive species. The high virulence of the parasite for Montana black-spotted cutthroat trout and Atlantic salmon may account in part for

the lack of success of efforts by the Oregon State Game Commission to establish these species of fish in streams within the enzootic area.

It is widely accepted principle that the pathogenicity of a parasite usually will be reduced with increasing duration of the host-parasite relationship. The results of my experiments appear to be generally in accord with this principle, which, therefore, may explain the differences in sensitivities of various salmonid fishes to N. salmincola. Atlantic salmon, Montana black-spotted cutthroat trout, kokanee, and their parent stock, sockeye salmon, except sockeye salmon in the Columbia River that are destined for streams east of the Cascade Mountains, do not occur naturally in the enzootic area of the "salmon poisoning" fluke (Snyder, 1940; Oregon State Game Commission, 1947; Carl, Clemens and Lindsey 1959). The other species tested occur naturally in the enzootic area. Thus, Atlantic salmon, Montana black-spotted cutthroat trout, and kokanee should be the most sensitive of the tested species to the effects of the parasites. The genus Oncorhynchus is more recent in origin than Salmo, having evolved from the latter in the western Pacific Ocean (Neave, 1958). Oncorhynchus presumably became established in the enzootic area later than did Salmo. Coastal cutthroat trout, therefore, should be more resistant to the parasite than coho salmon. The rainbow trout used in my studies were hatched from eggs obtained from brood stock maintained at a hatchery within the enzootic area. The history of this stock is not completely known, and therefore, it is difficult to speculate as to the significance of the relative

resistance of the rainbow trout to the infection. This rainbow trout stock came to Oregon from Utah approximately 30 years ago and thus this species should be as sensitive to the effects of the parasite as the other exotic species tested; however, the rainbow trout are more resistant than the non-native species of fish. This may be explained either as a result of selection or genetic contamination of the stock by either intentional or accidental interbreeding with trout native to the enzootic area. It is known that the rainbow trout stock tested was interbred with native rainbow trout approximately 25 years ago. However, factors other than previous contact of rainbow trout with the parasite may be involved in determining the relative resistance of this fish species.

Under the conditions of my experiments, the parasites killed fish during the time of their penetration and migration. The surviving fish may be weakened physiologically, as suggested by Wood and Yasutake (1956), during the later stages of infection when the parasites are encysted. Thus, the growth or swimming ability of such fish may be impaired.

Whether fish in nature encounter during a short period of time the numbers of cercariae that I used is not known. It is possible that fish can tolerate daily exposure to low numbers of parasites over a period of time, as stated by Farrell and Lloyd (1962), and thus accumulate large numbers of parasites that if acquired within 24 hours would be lethal. This would explain the large numbers of parasites found by Simms, Donham and Shaw (1931) and Simms et al (1931) in naturally infected fish. It is also

likely that exophthalmia is a result of chronic exposure of fish to low numbers of parasites, since I, in agreement with Bennington and Pratt (1960) and Farrell and Lloyd (1962), did not observe this condition.

The pathogenesis of the infection is not completely understood. It is probable that N. salmincola cercariae secrete proteolytic enzymes to aid in tissue migration, and high enzyme concentrations may be toxic to fish. The more resistant species of fish may have evolved natural antibodies to these enzymes. Such antibodies would be absent in the most sensitive species of fish.

Gebhardt (1966) found O. silicula snails infected with N. salmincola, in brackish water having a maximum salinity of 10 ppt. My finding infected snails in brackish water having a maximum salinity of 20 ppt shows that this freshwater snail and N. salmincola have adapted to or retained a tolerance for relatively high salinities. The only known published reports on the natural occurrence of other freshwater mollusks in saline water are those of Karpevich (in Zhadin, 1965) and Segerstråle (1957). Karpevich found species of the freshwater bivalve, Dreissena in brackish water in the Caspian Sea. Segerstråle reported the occurrence of the snails, Lymnaea peregra, Bithynia tentaculata, and Theodoxus fluviatilis, and the bivalves Anodonta cygnea and Dreissena polymorpha in the Baltic Sea.

This is the first reported study on the salinity tolerance of cercariae from a freshwater snail. Previous workers (Stunkard and Shaw, 1931; Rees, 1948; and Sindermann and Gibbs, 1953) have used cercariae from marine snails. Stunkard and Shaw (1931) stated that

a study on the effects of diluted seawater on freshwater cercariae was in progress at that time. The results of this study to my knowledge have not been published. My results show that N. salmincola cercariae can emerge from snails held in a salinity as high as 20 ppt. A salinity at which cercariae stopped emerging was not determined; however, it would probably not be much abcve 20 ppt because at this salinity few cercariae emerged. The optimum salinities for survival of cercariae from snails from freshwater and from brackish water were between 2 and 4 ppt, and 2 and 6 ppt, respectively. Large numbers of infected snails could not be obtained from brackish water and this precluded a more precise determination of the optimum survival salinity for cercariae from these snails. The findings of Shaw (unpublished) that N. salmincola cercariae live longer at low salinities than in freshwater agree with mine. He reported that cercariae survived (number not given) for 45 and 41 hours in 8 ppt sodium chloride and tap water, respectively. I found that 50% of the cercariae survived for 45 and 25 hours at 7 ppt salinity and in freshwater, respectively. The reason why cercariae survived longer in water having a low salinity than in freshwater may be explained by the findings of Picken (1937) who worked with the snail, Limnaea (sic) peregra. He reported that the vapor pressure of the snail blood was equivalent to a solution of sodium chloride at ca. 4.3 ppt salinity.

The results of my study suggest that N. salmincola cercariae have adapted to or retained a tolerance for saline conditions because they are able to emerge, survive, and penetrate fish at a

salinity as high as 20 ppt. The finding that cercariae from snails from brackish water lived longer at salinities above 12 ppt than cercariae from snails from freshwater suggests some physiological adaptation by the parasite to saline conditions.

If fish can be infected in brackish water in nature, as I have shown they can be in the laboratory, then possibly adult salmon that stray into brackish water within the enzootic area could become infected. If some of these fish spawned in streams outside the enzootic area then the disease agent could be carried to definitive hosts in new areas. Another implication from the finding that fish can be infected in saline water is that a lethal parasite dosage could be established in young fish if they remained in brackish water for extended periods of time, or in young fish reared in brackish impoundments having infected snails.

BIBLIOGRAPHY

Bennington, Edwin E. 1951. The life history of the salmon poisoning fluke Troglotrema salmincola (Chapin). Ph.D. Thesis. Corvallis, Oregon State University. 51 numb. leaves.

Bennington, Edwin E. and Ivan Pratt. 1960. The life history of the salmon-poisoning fluke, Nanophyetus salmincola (Chapin). Journal of Parasitology 46: 91-100.

Burns, William C. 1961. Six virgulate xiphidiocercariae from Oregon, including redescriptions of Allassogonoporus vespertilionis and Acanthatrium oregonense. Journal of Parasitology 47: 919-925.

Carl, G. C., W. A. Clemens and C. C. Lindsey. 1959. The freshwater fishes of British Columbia. 3d ed. Victoria, B. C. 192 p. (British Columbia Provincial Museum. Handbook no. 5)

Ching, Hilda Lei. 1957. The morphology of Oxytrema silicula (Gould). Master's thesis. Corvallis, Oregon State University. 60 numb. leaves.

Farrell, R. K. and M. A. Lloyd. 1962. The life cycle of the salmon poisoning fluke. In: Science in Alaska: Proceedings of the 12th Alaskan Science Conference. College, Alaska, American Association for the Advancement of Science, Alaska Division. p. 104-107.

Gebhardt, Gary Alan. 1966. Studies on the molluscan and fish hosts of the "salmon poisoning" fluke, Nanophyetus salmincola (Chapin). Master's thesis. Corvallis, Oregon State University. 72 numb. leaves.

Gebhardt, Gary Alan, R. E. Millemann, S. E. Knapp and P. A. Nyberg. 1966. "Salmon Poisoning" disease. II. Second intermediate host susceptibility studies. Journal of Parasitology 52: 54-59.

Neave, F. 1958. The origin and speciation of Oncorhynchus. Transactions of the Royal Society of Canada 52: 25-39.

Oregon State Game Commission. 1947. A report of fisheries investigations in Oregon coastal streams south of the Columbia River and exclusive of the Umpqua River. [Salem]. 79 p. (Mimeographed Report)

Philip, C. B. 1955. There's always something new under the "parasitological" sun (the unique story of helminth-borne salmon poisoning disease). Journal of Parasitology 41: 125-148.

Picken, L. E. R. 1937. The mechanism of urine formation in invertebrates. II. The excretory mechanism in certain molluscs. The Journal of Experimental Biology 20-34.

Rees, Gwendolen. 1948. A study of the effect of light, temperature and salinity on the emergence of Cercaria purpurae Lebow from Nucella Lapillus (L). Parasitology 38: 228-242.

Segerstråle, Sven G. 1957. Baltic Sea. In: Treatise on marine ecology and paleoecology, vol. 1, ed. by Joel W. Hedgepeth. Washington, D. C. p. 751-800. (Geological Society of America. Memoir 67)

Shaw, J. N., B. T. Simms and O. H. Muth. 1934. Some diseases of Oregon fish and game and identification of parts of game animals. Corvallis. 23 p. (Oregon. State Agricultural College. Station Bulletin no 322).

Simms, B. T. 1933. Pathogenicity of metacercariae of Nanophyetus salmincola, Chapin, for fish hosts. Journal of Parasitology 19: 160.

Simms, B. T., C. R. Donham and J. N. Shaw. 1931. Salmon poisoning. American Journal of Hygiene 13: 363-391.

Simms, B. T., C. R. Donham, J. N. Shaw and A. M. McCapes. 1931. Salmon poisoning. Journal of the American Veterinary Medical Association 78: 181-195.

Sindermann, Carl J. and Richard F. Gibbs. 1953. A dermatitis-producing schistosome which causes "clam diggers itch" along the central Maine coast. Augusta. 20 p. (Maine Department of Sea and Shore Fisheries Research bulletin no. 12)

Sinitsin, D. F. 1930. Contribution to the life history of the salmon-poisoning fluke of dogs, Nanophyetus salmincola (Chapin). Journal of Parasitology 17: 57-58.

Snyder, J. O. 1940. The trouts of California. California Fish and Game 26: 96-138.

Stunkard, H. W. and C. Ruth Shaw. 1931. The effect of dilution of sea water on the activity and longevity of certain marine cercariae, with descriptions of two new species. Biological Bulletin 51: 242-271.

Ward, H. B. and J. F. Mueller. 1926. A new pop-eye disease of trout-fry. Archiv für Schiffs-und Tropen-hygiene. 30: 602-609.

Wood, E. M. and W. T. Yasutake. 1956. Histopathology of fish. II. The salmon-poisoning fluke. Progressive Fish-Culturist 18: 22-25.

Zhadin, V. I. 1965. Molluscs of fresh and brackish waters of the USSR. Jerusalem, Israel Program for Scientific Translations. 368 p. (Keys to the fauna of the U.S.S.R., no. 46)